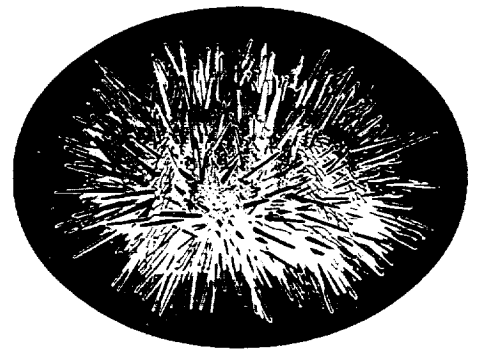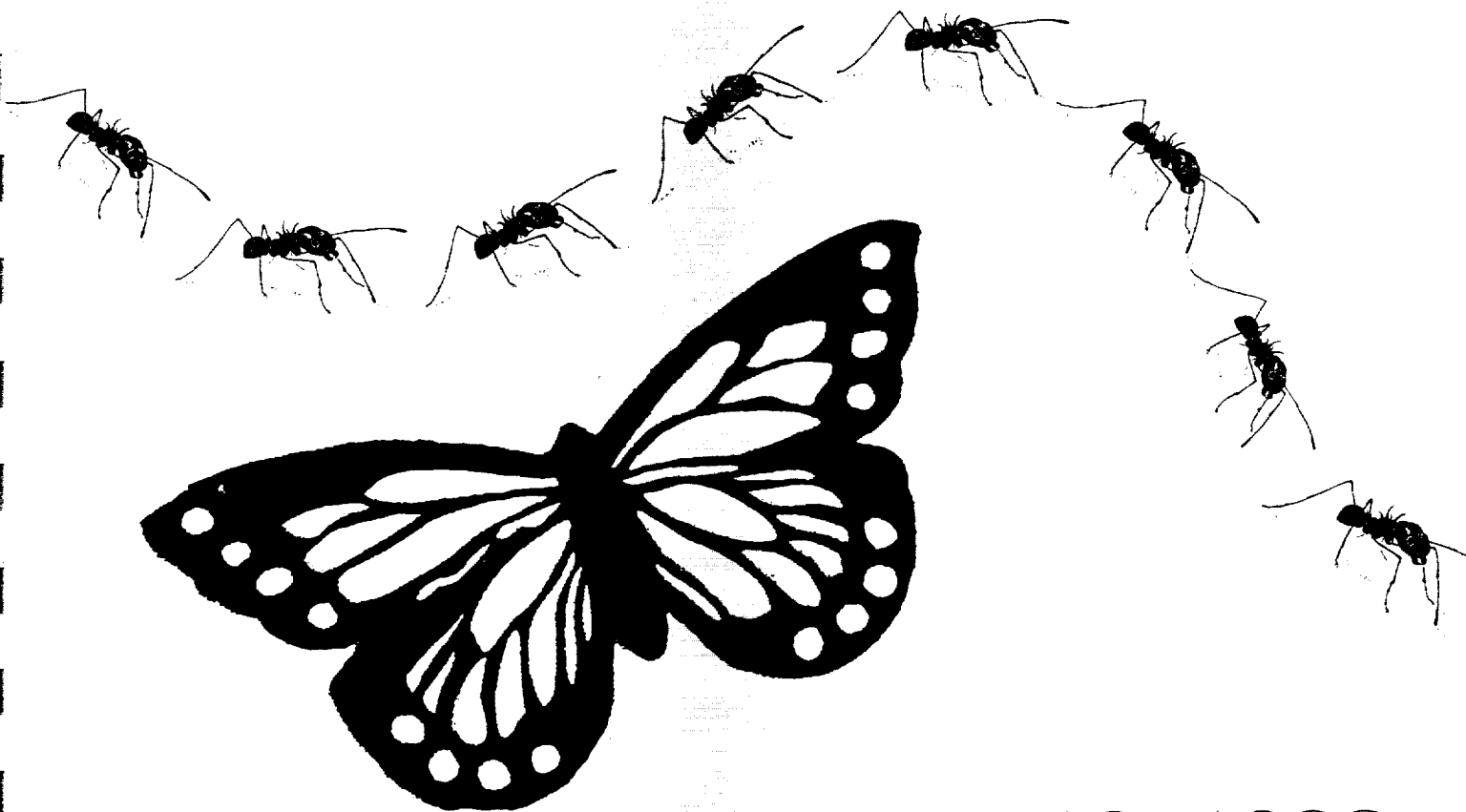# Developmental Gene Regulation and Mechanisms of Evolution

A workshop sponsored by
the Center for Advanced Studies
in the Space Life Sciences at
the Marine Biological Laboratory

June 10 - 13, 1998

Meigs Room, Swope Center
Marine Biological Laboratory
Woods Hole, Massachusetts

The Marine Biological Laboratory and the National Aeronautics and Space Administration have established a cooperative agreement with the formation of a Center for Advanced Studies in the Space Life Sciences (CASSLS) at the MBL. This Center serves as an interface between NASA and the basic science community, addressing issues of mutual interest.

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DEVELOPMENTAL GENE REGULATION AND MECHANISMS OF EVOLUTION

JUNE 10 - 13, 1998
MEIGS ROOM, SWOPE
MARINE BIOLOGICAL LABORATORY, WOODS HOLE, MA

NOTE :   Coffee/tea will be available throughout the day at the back of the room. Please help yourselves.

Wednesday, 10 June

7:00 am - 8:30 am	BREAKFAST	Swope, Main Dining Room

9:15 am   Welcome/Introduction - Lenny Dawidowicz, Eric Davidson

9:30 am - 12:00 noon   **MICHAEL AKAM'S LABORATORY**

Miodrag Gbric   Evolution of pattern formation in insects: Radical changes in development are associated with switch in the life history

Marion Rozowski   The role of *Hox* genes in establishing segment-specific traits in the legs of *Drosophila*

12:00 noon   LUNCH   Swope, MDR

1:30 pm - 4:00 pm   **MARTY SHANKLAND'S LABORATORY**

Elaine Seaver & Marty Shankland — Investigation of potential cell-cell interactions during early stages of segment formation in the leech embryo

Marty Shankland & Ashley Bruce — What do bilaterian 'head genes' tell us about the evolutionary origin of the bilaterian body plan?

Terri A. Williams & Marty Shankland — Segment and limb morphogenesis in primitive crustaceans

4:30 pm - 7:00 pm   **GARY RUVKUN'S LABORATORY**

Sarah B. Pierce & Gary B. Ruvkun — Identification of a putative DAF-2 insulin-like ligand

Brenda Reinhart, Frank Slack and Gary Ruvkun — The *C. elegans* heterochronic gene *let-7* encodes a small RNA with antisense complementarity to the 3'UTRs of other heterochronic genes

Catherine A. Wolkow & Gary B. Ruvkun — Insulin regulation of diapause in *C. Elegans*: Conservation or acquisition of insulin function?

7:00 pm   MIXER   Swope Terrace

7:30 pm   DINNER   Swope, MDR

Thursday, 11 June

7:00 am - 8:30 am	BREAKFAST	Swope, MDR

| 9:30 am - 12 noon | **JOEL ROTHMAN'S LABORATORY** | |

| 12 noon | LUNCH | Swope, MDR |

| 1:30 pm - 4:00 pm | **ANDRÉ ADOUTTE'S LABORATORY** | |

Guiallume Balavoine — The ancestry of the Hox cluster

Nicolas Lartillot & Olivier Lespinet — A comparative approach of mesoderm determination

Renaud de Rosa   Brachipod Hox genes and metazoan phylogeny

| 4:30 pm - 7:00 pm | **SEAN CARROLL'S LABORATORY** | |

David L. Lewis, Carig Brunetti, David N. Keys, Georg Halder, Victoria Kassner, Jane Selegue, Stephen Higgins, & Sean B. Carroll — Ectopic expression of transgenes in butterfly imaginal wing discs using recombinant Sindbis virus

John R. True & Sean B. Carroll — Genetics and evolution of Drosophila melanin patterning

Scott Weatherbee, Georg Halder, Angela Sebring, Jayne Selegue, H. Frederick Nijhout & Sean Carroll — Ubx regulation in the development and evolution of insect hindwings

| 7:00 pm | MIXER | Swope Terrace |
| 7:30 pm | DINNER | Swope, MDR |

Friday, 12 June

7:00 am - 8:30 am	BREAKFAST	Swope, MDR
9:30 am - 12:00 noon	**JAMES POSAKONY'S LABORATORY**	
12:00 noon	LUNCH	Swope, MDR
1:30 pm - 4:00 pm	**MICHAEL LEVINE'S LABORATORY**	

David N. Keys, David L. Lewis, Jane Selegue, Julie Gates, Lisa V. Goodrich, & Sean B. Carroll — Redeployment of known signalling pathways in the development of butterfly eyespots

4:30 pm - 7:00 pm	**NORIYUKI SATOH'S LABORATORY**	

Nori Satoh, Yutaka Satou, Michio Ogasawara & Hiroki Takahashi — Development and evolution of ascidians

7:00 pm	MIXER	Swope Terrace
7:30 pm	BANQUET	Swope, MDR

Saturday, 13 June

7:00 am - 8:30 am	BREAKFAST	Swope, MDR

9:30 am - 12:00 noon   **NADIA ROSENTHAL'S LABORATORY**

Nadia Rosenthal   Genetic regulation of muscle patterning

12:00 noon	LUNCH	Swope, MDR

1:30 pm - 4:00 pm   **GREGORY WRAY'S LABORATORY**

Ehab Abouheif   The evolutionary and developmental origins of wing polymorphism in ants

Alexandra Bely & Gregory A. Wray — Evolution of regeneration and asexual reproduction in annelids

Chrisopher Lowe   Genetic recruitment of body patterning genes and early life histroy diversity in echinoderms

Gregory A. Wray   New roles for old genes during echinoderm evolution

4:30 pm - 7:00 pm   **ERIC DAVIDSON'S LABORATORY**

R. Andrew Cameron, Cesar Arenas-Mena, Pedro Martinez & Eric Davidson — Ebryogenesis without expression of the Hox cluster: Transcripts of most Hox genes appear only in adult body plan formation in the sea urchin larva

Eric Davidson   1. A new overview of specification in the early development of the sea urchin, a typical Type 1 embryo
2. Immune system evolution in the deutorestomes

Kevin J. Peterson, R. Andrew Cameron, Kunifumi Tagawa, Nori Satoh & Eric Davidson — Evolution of animal body plans: A comparative gene expression approach to mesodermal patterning in basal deuterostomes

Chiou-Hwa Yuh & Eric Davidson — Developmental Cis-regulatory functions of Otx and contiguous binding sites in the sea urchin embryo

7:00 pm	MIXER	Swope Terrace

7:30 pm	DINNER	Swope, MDR

CONTENTS

CONTENTS CONTINUED

pupation, expression of a sensory organ precursor marker (*neuralized* -lacz) is fading from the two precursor cells and disappears completely. Preceding this event, Ubx protein is found at high levels in the ventral anterior distal tibia in the third leg where the apical bristle is located; moreover it is found at high levels in the second order precursors of this bristle compatible with a direct effect of Ubx on the interception of the development of this bristle.

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INVESTIGATION OF POTENTIAL CELL-CELL INTERACTIONS DURING EARLY STAGES OF SEGMENT FORMATION IN THE LEECH EMBRYO

Elaine Seaver
& Marty Shankland

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University of Texas
Austin, TX

Developmental Gene Regulation and Mechanisms of Evolution

The decision of a cell to adopt a particular fate during embyrogenesis is the result of the relative influence of extrinsic cues versus inherited intrinsic information. We are investigating the potential role that cell interactions play during early stages of segment formation in the leech. During segment formation in *Drosophila*, the basic segmental unit consists of 4 rows of cells in which critical cell interactions along the A/P axis establish segment polarity. In *Drosophila* this specification of segment polarity occurs in the context of a variable cell lineage. In leech, the segmental tissues are generated from iterated asymmetric divisions of a group of stem cells. Each teloblast produces a chain of primary blast cells which will then divide to produce a clone of roughly 70 cells comprising a single segmental repeat. The descendant clones as well as the early divisions of the primary blast cells have been well characterized making the leech embryo a strong experimental system for examining cell interactions at the single cell level. We wanted to determine whether there are critical cell interactions between primary blast cells or if cell interactions become important in determining cell fate once primary blast cells have divided. After primary blast cells have divided at least once there is potential for segment polarity to become established because adjacent cells are no longer the same. We have utilized a laser ablation system in which single cells can be killed, removing potential critical interactions. In initial experiments, interactions between primary blast cells in the O and P teloblast lineages were examined before they undergo any cell divisions. Interactions between primary blast cells were examined in both the anterior to posterior and posterior to anterior directions along the body axis. Results thus far indicate that the ability of blast cells to produce the appropriate segmental complement of differentiated cells is not dependent upon cell interactions between primary blast cell clones, i.e. the fundamental repeating unit of the leech's segmental body plan appears to develop with a large degree of autonomy.

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WHAT DO BILATERIAN 'HEAD GENES' TELL US ABOUT THE EVOLUTIONARY ORIGIN OF THE BILATERIAN BODY PLAN

Marty Shankland
& Ashley Bruce

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The orthodenticle orthologue Lox22-Otx was isolated from the leech *Helobdella triserialis*. *In situ* hybridization reveals that embryonic expression of Lox22-Otx RNA is primarily restricted to an unsegmented head domain, including tissues in the foregut, surface ectoderm, and the head ganglion of the central nervous system. Patterns of head expression form concentric rings about the stomadeum, and mark tissue domains that exhibit discrete behaviors during later morphogenesis and differentiation. Expression was also observed in 1-2 bilateral pairs of neurons in each segmental ganglion or neuromere of the body trunk. The largely head-specific expression of Lox22-Otx in this annelid supports data from two other bilaterian phyla (arthropods and chordates) in suggesting the existence of a genetically defined head/trunk distinction. We suggest that this head/trunk distinction is a synapomorphy of the Bilateria as a whole, and that it reflects some sort of regional or temporal distinction within the body plan of an early bilaterian ancestor. A model will be discussed in which the bilaterian body plan is derived from a radially organized prebilaterian ancestor by the addition of a discrete 'trunk domain' that was situated anisotropically with respect to the axis of radial symmetry. The model suggests that the spatial restriction of 'head gene' expression in most Bilateria reflects a failure of these genes to be coopted into the early patterning of the trunk domain. Our model also portrays the anteroposterior axis of Bilateria as being novelty associated with trunk elongation. If this is the case, then AP organization would have been secondarily imposed onto the bilaterian head domain by its functional integration with the elongating trunk domain.

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SEGMENT AND LIMB MORPHOGENESIS IN PRIMITIVE CRUSTACEANS

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& Marty Shankland

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Austin, TX

Developmental Gene Regulation and Mechanisms of Evolution

Because of our dependence on model systems in developmental biology the generality of our theories of developmental mechanisms is uncertain. I will discuss development in branchiopods, a group of crustaceans that bear a long series of similar limbs on the thorax, and draw comparisons from these to known model systems. Segmentation is relatively slow in branchiopods; segments are formed over a number of days during a free living larval period. Segments are formed in an anterior/posterior sequence within a morphologically undifferentiated field of cells. Also, in distinction to *Drosophila* and many other arthropods, virtually all of the cells in the ventral region of the limb-bearing segments are destined to form the limb. Thus, the early morphogenesis of segments and limbs are very tightly linked processes. I will discuss two aspects of these processes: 1) the cellular mechanisms responsible for creating the field of cells that subsequently form segments and 2) the early morphogenesis of limbs on those segments. Patterns of incorporation of BrdU demonstrate that high levels of mitosis do not occur in a subterminal growth zone, as would be predicted if teloblastic stem cells were present, but rather occur just posterior to the segmented region of the trunk. I am currently using cell lineage tracing to follow the fates of cells in the putative growth zone. Finally, based on patterns of limb morphogenesis in a number of branchiopod orders, I propose that it is difficult to draw a strict one to one homology between the branches of branchiopod limbs and the standard two branched limb of crustaceans or the unbranched *Drosophila* leg. Thus claims of homology of patterning mechanisms, based solely on comparisons of regulatory gene expression patterns, may not be well grounded in a plausible morphogenetic context.

IDENTIFICATION OF A PUTATIVE DAF-2 INSULIN-LINKED LIGAND

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& Gary B. Ruvkun

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In the nematode *Caenorhabditis elegans* the activity of an insulin-like signaling pathway is required for reproductive development and normal adult lifespan. Wild-type animals enter the developmentally arrested dauer stage in response to high levels of a secreted pheromone. It is known that neurosecretory cells are required for this process. Mutants in DAF-2, a homolog of insulin and insulin-like growth factor I (IGF-I) receptors (1), arrest at the dauer stage when grown at non-permissive temperatures and have increased longevity when shifted to non-permissive temperatures in early adulthood. DAF-2 is likely to be regulated by an insulin-like ligand. By searching the worm database, we determined that the *C. elegans* genome contains at least eight genes predicted to encode insulin-like peptides. The predicted proteins contain the invariant cysteines and, in some cases, predicted proteolytic cleavage sites that are hallmarks of the insulin superfamily. Because DAF-2 is likely to encode the only insulin/IGF-I receptor, it is perhaps surprising that there are so many insulin family members in *C. elegans*. These genes may function redundantly to activate the DAF-2 receptor or, alternatively, some may be activators and others inhibitors of DAF-2. The gene most closely related to the vertebrate insulins, which is on cosmid F13B12, was chosen for further analysis. The expression pattern of F13B12(ins) was determined using a GFP transgene. F13B12(ins)::GFP is expressed only in neurons, including a pair of pharyngeal neurons, several pairs of head neurons, and three tail neurons. This is consistent with the neuronal expression of insulin-like peptides in several other invertebrates and with the hypothesis that F13B12(ins) acts in the neurosecretory signaling system controlling dauer formation. To investigate whether F13B12(ins) can play a role in dauer formation, the entire F13B12(ins) genomic region was amplified by PCR and used to construct transgenic worms, with the goal of overexpressing F13B12(ins). We hypothesized that, if F13B12(ins) is the DAF-2 ligand, overexpression of F13B12(ins) might suppress the dauer-constitutive (Daf-c) phenotype of weak *daf-2* mutants or mutants in the parallel *daf-7* transforming growth factor-ß (TGF-ß) pathway, which synergizes with the *daf-2* insulin-like signaling pathway (2) . The F13B12(ins) transgene was unable to suppress either of two non-null *daf-2* alleles (*e1370* and *e1365*). Although these are non-null alleles, there may not be enough extra

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ligand produced in the F13B12(ins) transgenic worms to overcome the effect of the impaired DAF-2 receptor. However, the F13B12(ins) transgene maternally suppressed the Daf-c phenotype of *daf-7(e1372)*, which encodes a TGF-ß-like ligand (3). Mutations in the *unc-64* gene, which encodes a homolog of syntaxin (4), a component of the neurosecretory apparatus, have a Daf-c phenotype at 27°C and extended adult life span at 20°C. The fact that these phenotypes are suppressed by mutations in *daf-16*, which also suppress *daf-2* mutations, suggests that this may be the result of decreased secretion of the DAF-2 ligand (J. Thomas, personal communication). *unc-64(e246); F13B12(ins)* worms briefly arrest as dauers at 27°C and then, unlike *unc-64(e246)* control worms, rapidly recover and develop into fertile adults. The expression pattern of F13B12(ins)::GFP and the ability of the F13B12(ins) transgene to suppress dauer formation and promote dauer recovery in *daf-7(e1372)* and *unc-64(e246)* mutants, respectively, are consistent with a role for F13B12(ins) in the neurosecretory signaling system that regulates dauer formation. We are continuing to investigate the ability of F13B12(ins) to affect dauer formation and aging and are exploring candidate mutations in the F13B12 region.

Developmental Gene Regulation and Mechanisms of Evolution

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THE *C. ELEGANS* HETEROCHRONIC GENE *LET-7* ENCODES A SMALL RNA WITH ANTISENSE COMPLEMENTARITY TO THE 3'UTRs OF OTHER HETEROCHRONIC GENES

Brenda Reinhart
Frank Slack
& Gary Ruvkun

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Coordination of the timing and sequence of developmental events is a problem common to all higher organisms. When the timing of a developmental decision is altered relative to others in the same organism, the resulting phenotype is termed heterchronic. Heterochronic mutations have been identified in C. *elegans*, *Drosophila*, and maize. However, the only system where multiple heterochronic genes have been placed in a genetic pathway is in C. *elegans* (1). A key player in this hierarchy is *lin-14* (**lineage**), a novel nuclear protein of unknown function that is required for the proper temporal progression of postembryonic development. Mutations in *lin-14* perturb the normal sequence of developmental stages. Wild type animals proceed from embryogenesis through four larval stages (L1-L2-L3-L4) punctated by molts before reaching adulthood. The V cells of the lateral hypodermis, which secrete new cuticle with each larval molt, divide at each larval stage but exit the cell cycle and terminally differentiate at the adult molt. In *lin-14(null)* mutations, the L1 V divisions are absent, the other larval divisions occur precociously, and terminal differentiation and adult specific cuticular structures called alae appear precociously. Gain-of-function mutations in *lin-14* have the opposite phenotype; they reiterate V cell divisions and delay terminal differentiation.

A screen for suppressors of the heterochronic defects of *lin-14(lf)* identified a viable allele of *let-7* (**lethal**). This partial loss of function allele, *let-7(n2853)*, has a delay in the appearance of adult alae, presumably due to a delay in the terminal differentiation of the V cells of the lateral hypodermis (2). This hypodermal phenotype is reminiscent of loss-of-function alleles of the heterochronic gene *lin-4*, which encodes a small antisense RNA (3). In addition, *let-7(n2853)* is partially temperature sensitive for a bursting at the vulva at the L4 molt, which is fully penetrant in the canonical allele *let-7(mn122)*. Both the alae defect and vulval bursting are suppressed by loss of function mutations in the precocious heterochronic genes *lin-14* and *lin-28*.

We have cloned *let-7* by phenotypic rescue. The minimal rescuing genomic fragment is not predicted to produce a protein. cDNA library probings, RT-PCR, and Nothern blots have failed to detect any spliced messages, and cloning of the C. *briggsae let-7* genomic

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region has revealed no compelling protein conservation. However, PAGE Northern blots have identified a small (23-24nt) transcript in wild-type that is reduced in *let-7(n2853)*, a point mutation predicted to be in the RNA, and absent in *let-7(mn112)*, a deletion.

Considering the similarity of the *let-7* phenotype to *lin-4*, we are investigating the possibility that the *let-7* RNA acts in an antisense mechanism similar to the *lin-4* RNA. *lin-4* post-transcriptionally regulates the levels of LIN-14 and LIN-28 through pairing with complementary sequences in their 3'UTRs (4). We have found multiple sequences complementary to the *let-7* RNA in the 3'UTR of *lin-14* and a single site in the 3'UTR of *lin-28*. The structure of the proposed RNA duplexes is quite similar to those formed by *lin-4*. An 8bp region of perfect complementarity exists in all sites, then a loop region leads to another complementary region which has a more variable sequence than the first. The 8bp perfect match would be disrupted by the *n2853* point mutation. We are currently testing whether these genes are regulated by *let-7* via these complementary 3'UTR sequences. In addition, we are analyzing the expression profile of *let-7* to determine whether *let-7* is acting at the same time as *lin-4*, perhaps in a redundant role, or whether *let-7* is acting at a different stage of development to refine the expression of LIN-14 and LIN-28.

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(4) Wightman, Ha, and Ruvkun, 1993, Cell 75, 855-862.

Developmental Gene Regulation and Mechanisms of Evolution

Insulin regulation of diapause in C. elegans: Conservation or acquisition of insulin function?

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In response to environmental cues, the nematode C. elegans, enters developmental diapause and becomes a dauer larva. The physiology of the dauer larva is optimized for survival under hostile environmental conditions such as scare food or overpopulation (reviewed in (1)). Dauer larvae cease feeding and are encased in a thick cuticle. In addition, dauer larvae have extended life spans which are correlated with high levels of catalase and superoxide dismutase. These physiological adaptations enable long term survival of dauer larvae. Dauer larvae recover to form normal reproductive adults in response to cues of an improved environment, such as the presence of food.

C. elegans dauer formation is controlled by an insulin-like signaling pathway. Dauer entry is prevented by the action of two genes, *daf-2* and *age-1* (2,3,4). *daf-2* encodes an insulin receptor-like protein and *age-1* encodes a homolog of the p110 catalytic subunit of mammalian phosphatidylinositol 3-kinase (PI(3)K) known to act downstream of the insulin receptor in vertebrates (4,5). Mutations in either *daf-2* or *age-1* cause animals to constitutively form dauer larvae, regardless of environmental cues. The activity of a third gene, *daf-16* , is required for dauer formation in *daf-2* or *age-1* mutants (2,3,4). *daf-16* encodes a forkhead family transcription factor (6). The genetic interaction between *daf-2* and *daf-16* shows that *daf-16* is a downstream target of insulin in C. elegans.

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Has insulin function been conserved between C. elegans and vertebrates, or have functions of insulin changed during evolution? Vertebrate insulin performs a well-characterized role in metabolic control. Insulin production is stimulated in response to feeding and activates metabolic outputs in target tissues. Ligand binding to insulin receptors in target tissues causes activation of downstream signaling cascades. PI(3)K is a major effector of insulin receptor signaling and, in turn, activates the protein kinase, Akt/PKB (reviewed in (7)). Some well known physiological outputs of vertebrate insulin include increased glucose uptake by adipocytes and glycogen synthesis in liver. However, vertebrate insulin is not known to directly regulate developmental switches that might be similar to C. elegans dauer formation.

We are interesting in determining if insulin performs different functions in *C. elegans* and vertebrates. As a first step towards answering this question, we have identified components of the *C. elegans* insulin pathway using forward and reverse genetic approaches. In vertebrates, PI(3)K functions as a heterodimeric enzyme composed of a catalytic subunit and a regulatory subunit. *age-1* is homologous to vertebrate PI(3)K catalytic subunits (4). Using the published *C. elegans* genomic sequence, a homolog of the PI(3)K regulatory subunit was identified. The candidate *C. elegans* p55 shares the architecture of vertebrate PI(3)K regulatory subunits, containing two SH2 domains flanking an inter-SH2 domain. The amino terminal SH2 domain of the *C. elegans* p55 homolog is more closely related to SH2 domains from vertebrate p55 proteins than to the SH2 domains from other SH2-domain containing proteins, such as Src or Vav. We are currently investigating the function of the *C. elegans* p55 homolog. In addition, others in the Ruvkun lab have shown that Akt is a downstream target of insulin signaling in *C. elegans* (8). Together, these findings show that *C. elegans* and vertebrate insulin couples to conserved downstream targets.

We are beginning to identify and analyze potential physiological outputs of *C. elegans* insulin. Potential targets of *C. elegans* insulin include the known targets of vertebrate insulin, such as cellular glucose uptake pathways and metabolic enzymes. Identifying conserved and non-conserved insulin outputs will help to illuminate how insulin controls dauer formation in *C. elegans*.

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THE ANCESTRY OF THE HOX CLUSTER

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A complex of *Hox* genes was present in the last ancestor of all triploblast animals (Urbilateria). This cluster was already functioning in the patterning of the anterior-posterior axis of the animal. In contrast, there is until now no evidence for the existence of a Hox cluster in the cnidarians or the sponges, which have emerged earlier in the metazoan family tree. The cluster presumably appeared by gene duplication in the ancestors of the Bilateria.

A common view is that this process of gene duplication has more or less followed an increase of complexity of the body plan in Bilateria, passing through a four or five-gene stage when the nematodes diverged, to six or seven genes in the ancestor of the arthropods and vertebrates, to at least ten genes in the ancestor of the chordates and thirteen in the ancestor of the vertebrates.

A series of new discoveries challenge this view and raise the possibility that the whole cluster with numerous genes appeared in a single genetic radiation and that the history of the cluster since has been dominated by gene divergence and extinction.

- the 18S rDNA phylogeny of metazoans leads to the picture that the bulk of the bilaterian phyla is split into a double dichotomy giving three great superphyla, Deuterostomia, Ecdysozoa (including nematodes) and Lophotrochozoa (including brachiopods). The four-gene cluster in *C. elegans* must therefore be the remnant of a larger cluster. Comparison of the *Hox* genes sequences appears to confirm the position of some phyla in this three-branched tree of the Bilateria, notably brachiopods (R. de Rosa).

- the presence of several *Abd-B*-like genes in deuterostome phyla and of at least two of them in lophotrochozoans opens the possibility that several *Abd-B* genes were already present in the Urbilateria.

- some *Hox* genes of the fruitfly, which do not possess any homeotic function, are shown to derive from ancient *Hox* genes, rather than being recent duplicates of existing *Hox* genes. This is

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especially the case for *zerknüllt*, whose homologs in various arthropods (including basal insects) are quite similar to the vertebrate *Hox3* genes.

A fully developed ancestral cluster might have evolved in the context of the emergence of the Urbilateria body plan, maybe in association with the metameric segmentation.

**Developmental
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and
Mechanisms of
Evolution**

A COMPARATIVE APPROACH OF MESODERM DETERMINATION

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Metazoan body plans are so distinct from each other that they are not easy to reconcile into a robust phylogeny. In spite of this, morphological considerations, some of which bearing on very early developmental events such as the number of germ layers, the fate of the blastopore, have allowed classical embryologists to draw a broad scheme for a metazoan phylogeny, which does not seem to be fundamentally challenged by molecular data. This suggests that the concepts of germ layers and gastrulation are indeed significant in a comparative approach.

Mesoderm, as a *bona fide* germ layer, is the distinctive trait of triploblastic animals. Furthermore, at least in vertebrates and spiral cleaving protostomes, mesoderm determination is tightly coupled with dorso-ventral axis specification. These facts raise the question of whether the specification of a third germ layer is a homologous trait shared by bilateral animals. Some molecular markers for mesoderm are conserved between arthropods and vertebrates. But their expression patterns are not always pan-mesodermal (like *twist* in vertebrates), nor exclusively mesodermal, like *snail* which is expressed in all three germ layers. Indeed, these molecules belong to genetic cascades that are recruited to achieve similar regulatory functions in other aspects of development, and thus do not plead conclusively for mesoderm homology. We argue that one way to establish a frame for a robust comparative approach of that question would be to compare not only the expression patterns of genes involved in specific germ layers, but also the molecular mechanisms of the early inductive events leading to the restriction of these expression patterns to each germ layer.

In a first step, we wish to better understand the molecular aspects of mesoderm ontogeny in protostomes, and we have started a comparative analysis of the genetic cascades presumably involved in mesoderm specification in three protostomian species exhibiting spiral cleavage: the mollusc *Patella vulgata*, the annelid *Sabellaria alveolata* and the nemertine worm *Cerebratulus lacteus*. In these species, we isolated homologues of the *Drosophila twist, snail* and *decapentaplegic* for which we have indications that they are expressed as early as at the blastula stage in each of our three models. We will first study and compare the patterns of expression

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of these genes. Then, a dissection of the cis-acting sequences, monitored by microinjections of GFP fusions, will be performed. The comparative approach will bear on the variations in mesoderm determination between spiralians as well as on the question of the homology of mesoderm among triploblasts.

Developmental Gene Regulation and Mechanisms of Evolution

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The phylogenetic status of lophophorates (*i.e.* brachiopods, phoronids and ectoprocts (*i.e.* bryozoans), all groups sharing a tentacular organ named the lophophore) has long been debated on the basis of morphological and embryological data. The two most common hypotheses are the following:

- the lophophorates are deuterostomes, and are a sister group to echinoderms and chordates. A radial-like cleavage, deuterostomy and a three-part cœlom argue for this grouping;
- the lophophorates are protostomes, and within protostomes, they have more affinity to molluscs and annelids than to arthropods. The strongest argument for this is the existence in some species of a larval stage very similar to a trochophore.

18S rRNA-based molecular phylogenies have recently favored the latter hypothesis. A group called Lophotrochozoa (annelids, molluscs, lophophorates and a few other phyla) has therefore been proposed. It should be noticed that lophophorates do not always appear to constitute a monophyletic group, except for brachiopods and phoronids whose sister group relationship seems reliable.

Signatures within members of the Hox genes have already been used as phylogenetic markers. Among protostomes, the central class (Antp-like) Hox genes can roughly display two states: either the Antp/Ubx/abd-A genes discovered in arthropods, or the Lox5/Lox2/Lox4 genes identified in annelids, and later found in molluscs and nemertines. On the basis of sequence similarities, Antp and Lox5 are assumed to be orthologues. Whether Lox2 and Lox4 are orthologous to Ubx and abd-A respectively, or descend from one unique ancestral gene (named Ubd-A or Lox2/4) is not certain. However, due to some characteristic residues in the homeodomain, the Lox2-, Ubx-, Lox4- and abd-A-like can clearly be distinguished from each other. And what seems clear is that animals studied up to now display either one of the two associations: Ubx and abd-A or Lox2 and Lox4.

Using a degenerate PCR technique, we performed a screening for Hox genes on the inarticulate brachiopod *Lingula lingua*. This screening yielded the following results:

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- the central class Hox genes of the brachiopod comprise clear Lox2- and Lox4-like genes. This striking similarity with annelids strengthens the second phylogenetic hypothesis proposed above;

- the posterior class Hox genes (Homologous to Abd-B in arthropods and PG9-13 in chordates) comprise at least two genes, one of which is very similar to an annelid gene, the other to a nemertine gene. These results suggest that the last common ancestor to lophotrochozoans may have had two Abd-B-like genes.

A screening was also performed on the nematomorph *Gordius aquaticus* (a sister group to nematodes), which seems to have only a few, very divergent, Hox genes (4 were found). One is very arthropod Ubx-like, which would confirm the existence of the group Ecdysozoa, although the risk of cloning arthropod genes instead of nematomorph ones can not be ruled out due to the living habits of *G. aquaticus*. Orthology relationships with *Cœnorhabditis elegans* genes could not be solved.

N.B. part of the DNAs used in this study were kindly provided by Birgitta Winnepennickx.

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ECTOPIC EXPRESSION OF TRANSGENES IN BUTTERFLY IMAGINAL WING DISCS USING RECOMBINANT SINDBIS VIRUS

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The wings of different butterfly species are often adorned with unique color patterns. Patterns of gene expression of components of the *hedgehog* and *decapentaplegic* signaling pathways suggest these are involved in eyespot pattern formation. In order to understand the role of these pathways, we are investigating the potential of recombinant Sindbis viruses as a means to ectopically express transgenes in butterfly wing imaginal discs.

Recombinant Sindbis virus was prepared containing the gene encoding green fluorescent protein (GFP) under control of a duplicated viral subgenomic promoter. Larval and pupal stage butterflies (sp. *Precis coenia*) were injected with recombinant virus and then examined for GFP expression at various times after infection. In larval stage animals, GFP was detected in the fat bodies and nervous tissue. In pupal stage animals, GFP fluorescence was detected in nervous tissue and in wing imaginal disc cells. Importantly, no deleterious effects resulting from viral infection and transgene expression were observed on either the survivability of the animals or on the morphology of the wing scales and wing color patterns of infected adults. Infection of early pupal wing discs with recombinant Sindbis viruses expressing either an activated form of *cubitus interruptus* or the *Precis coenia* homolog of *decapentaplegic* results in the formation of an ectopic eyespot. These results are consistent with a model of eyespot determination involving both *hedgehog* and *decapentaplegic* signaling.

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GENETICS AND EVOLUTION OF DROSOPHILA MELANIN PATTERNING

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The black pigment melanin is an important component of adult insect cuticle and its patterns are highly variable in evolution. In order to shed light on the development of these patterns and to identify candidate genes for involvement in melanin pattern evolution we are analyzing the roles of several genes in the melanin synthesis pathway in *Drosophila melanogaster*. These include *Tyrosine Hydroxylase* (TH; encoded by the *pale* gene), *Dopa decarboxylase* (*Ddc*), and *Diphenol oxidase A2* (*Dox-A2*). Mitotic clonal analysis indicates that all three of these genes are required cell autonomously for melanin production in the adult abdominal stripes. We are using the GAL4/UAS ectopic expression system to determine which genes are sufficient for ectopic melanin production in adult wings. We present preliminary data showing that expression of UAS-TH causes a dominant negative lightening of the abdominal stripes and a low level of ectopic pigmentation in the intervein areas of the wing while UAS-DDC alone has no apparent effects on adult pigmentation. Expression of UAS-TH and UAS-DDC together enhances the wing phenotype and causes a variable suppression of the abdominal UAS-TH phenotype. Future directions include analysis of expression patterns of these genes in various Drosophila species and investigation of the role of wing veination in melanin patterning.

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UBX REGULATION IN THE DEVELOPMENT AND EVOLUTION OF INSECT HINDWINGS

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Arthropods and vertebrates are constructed of many serially homologous structures whose individual patterns are regulated by Hox genes. The Hox-regulated target genes and developmental pathways that determine the morphological differences of homologous structures within or between species are not known. In Drosophila, haltere, as opposed to wing, development is controlled by the *Ultrabithorax (Ubx) Hox* gene. We show that Ubx independently regulates the expression of selected genes that act at many levels of the wing-patterning hierarchy. These include the Wingless signaling protein and tiers of Wg- and Decapentaplegic-activated target genes that control morphological features that differ between the wing and haltere. We also analyze a remarkable *Precis coenia* mutant butterfly in which portions of the ventral hindwing pattern are transformed to that of the ventral forewing. We show that Ultrabithorax (Ubx) protein expression is lost from patches of cells on the developing hindwings of these mutants which correlates with changes in pigmentation, color pattern elements, and scale morphology. We found that the *Distal-less (Dll)* gene, which is deployed in a novel fashion in butterfly wings is regulated by Ubx. However, several genes that are regulated by Ubx in the *Drosophila* haltere are not repressed by Ubx in butterfly hindwings. Our findings suggest that as halteres evolved from the hindwings of a four-winged ancestor, regulatory interactions arose independently between Ubx and many wing patterning genes.

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REDEPLOYMENT OF KNOWN SIGNALLING PATHWAYS IN THE DEVELOPMENT OF BUTTERFLY EYESPOTS

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In animals, color pattern is a phenotype under extremely strong selective pressure. In butterflies, this has produced a wide variety of novel, and flexible, wing color patterns, including eyespots. Different species deploy eyespots with different sizes, pigments, numbers and positions. In order to address the developmental changes which must underlie the evolution of such diversity, we are investigating the mechanisms which produce these patterns.

Eyespots are determined using a prepattern, which creates potential eyespots in every veined compartment, and location specific signals, which produce foci in a subset of these positions. Homologs of *hedgehog* and *decapentaplegic* are transcribed in patterns correlating with the prepattern. Additionally, there is an increase in *hedgehog* transcription levels neighboring foci specific locations. *patched, cubitus interruptus* and *engrailed* express in these locations, but not other potential foci. These results suggest a model in which *hedgehog* signalling, regulated by prepattern and position specific information, determines the focus pattern elements. We are using ectopic expression with Sindbis virus to investigate this model further.

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Ascidians (subphylum Urochordata, class Ascidiacea) are ubiquitous marine animals. Since the turn of this century, ascidian embryos have served as an experimental system in developmental biology. The fertilized egg develops quickly into a tadpole larva (about 2,600 component cells), which consists of a small number of tissues including epidermis, central nervous system with two sensory organs, nerve (spinal) cord, endoderm, mesenchyme, notochord and muscle. Lineage of these embryonic cells is completely described up to the gastrula stage. In addition, recent cloning of various tissue-specific genes provides molecular probes with which to monitor differentiation of each type of tissues. These features serve an opportunity to study the mechanisms underlying the specification and pattern formation of the embryo. Furthermore, recent studies support the ascidian tadpole prototype for the ancestral chordates. Therefore, the development of the ascidian tadpole larva may provide clues concerning the origin and evolution of chordates.

Maternal genes with localized mRNA: The ascidian embryo shows a highly determinate mode of development, which may be dependent on prelocalized egg cytoplasmic determinants. We isolated cDNA clones for several maternal genes with localized mRNA. Because all of these mRNAs are localized in the posterior-vegetal cytoplasm of the egg and they later mark the posterior end of developing embryos, we named the genes *posterior end mark* (*pem*). Thus far we characterized cDNA clones for six *pems*; *pem*, *pem-2*, *pem-3*, *pem-4*, *pem-5*, and *pem-6*. *pem-3* encodes a polypeptide with KH domain and RING finger (a possible RNA-binding protein), and zygotic expression appears to play a role in the CNS formation.

 pem overexpression causes a loss of the anterior and dorsal structure of the larva. To deduce functional cascade of *pem*, we examined effect of lithium treatment on ascidian embryogenesis. We found that the deficiency induced by *pem* overexpression was rescued by lithium treatment. A candidate target of lithium is GSK-3b. We isolated an ascidian b-catenin gene, and confirmed that overexpression of b-catenin had effects similar to lithium treatment.

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Upstream and downstream of *Brachyury*: Recent studies have revealed that *Brachyury* play a crucial role in notochord formation in ascidian embryos. In collaboration with Mike Levines lab, we investigate genetic circuitry of ascidian *Brachyury*.

[Upstream] J. Corbo et al. (1997) identified a minimal, 434 bp enhancer from the *CiBra* (*Ciona Brachyury*) promoter region that mediates the notochord-restricted expression of reporter genes. This enhancer contains an ectopic (mesenchyme and muscle) repressor region, notochord activation region, and an ectopic activation region. On the other hand, *As-T* (*Halocynthia Brachyury*) contains only a notochord activation region in the enhancer. We attempted reciprocal injections of the fusion genes to examine common and uncommon mechanisms between the two species.

[Downstream] *CiBra* ectopic expression was induced by injection of a fusion gene construct in which the promoter of *Cifkh* was fused with *CiBra* coding sequence into *Ciona* eggs. This ectopic expression was used to isolate candidate *CiBra* downstream genes. Thus far, we have isolated about 500 cDNA clones for *CiBra* downstream genes, updated information of them being presented.

Endostyle and pharyngeal gill: In addition to notochord and nerve cord, pharyngeal gill and endostyle are structures shared by chordates. These organs may have arisen in their common ancestor with a shift to internal feeding for extracting suspended food from the water. In addition, the endostyle has functional homology to the vertebrate thyroid gland. We have been studying molecular mechanisms involved in the formation of these organs. As to the endostyle, we isolated and characterized cDNA clones for endostyle-specific genes, *HrEnds1* and *HrEnds2* from *Halocynthia*, and *CiEnds1*, *CiEnds2*, *CiEnds3*, and *CiEnds4* from *Ciona*. Interestingly, *HrEnds2*, *CiEnds1* and *CiEnds2* were expressed in zone 6, and the amino acid sequences of HrENDS2, CiENDS1 and CiENDS2 resembled each other. These zone-6-specific genes may be conserved among ascidian species, and therefore they provide useful probes for further analyses of molecular mechanisms involved in the endostyle development. We also isolated pharyngeal gill-specific genes, *HrPhG1* and *HrPhG2*. In addition, an ascidian *pax-1/9* gene is specifically expressed during the formation of this organ.

GENETIC REGULATION OF MUSCLE PATTERNING

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Our general research objective has been to identify the genetic pathways controlling the specification of skeletal muscle pattern. The myogenic phenotype is one of the most extensively characterized at the molecular level, and the system is ripe for linking the mechanisms that commit stem cells to the muscle lineage with the pathways that specify their patterning and subsequent diversification. A current interest of the laboratory is to characterize the molecular cues which establish patterns of muscle gene accessibility in the embryo. These cues are the key to an understanding of tissue remodeling in the adult, in response to injury and neuromuscular pathologies. Another area of research focuses on genetic pathways underlying the diversification of muscle fiber types during muscle development, and their selective loss during aging and neuromuscular disease. Most recently, our interest in tissue patterning has extended to the heart, where we have begun to characterize several regional gene regulation pathways, and have initiated an investigation into the role of retinoids in cardiac morphogenesis in the vertebrate embryo.

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The evolutionary and developmental origins of wing polymorphism in ants

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Morphological polymorphisms have independently evolved in several insect lineages, including ants and termites. These polymorphisms are associated with dramatic changes in life-history and social organization. The existence of a polymorphism in a species implies that there must have been important evolutionary changes in the regulatory pathways underlying these structures. Wing polymorphism among the different social castes in ants provides an excellent system to investigate this phenomenon. Currently, we are cloning, sequencing, and examining the expression of developmental genes known to be involved in *Drosophila* wing development from the ant *Pheidole morrisi*.

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EVOLUTION OF REGENERATION AND ASEXUAL REPRODUCTION IN ANNELIDS

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Although embryogenesis is the most familiar way of producing an adult, many animals can produce an adult phenotype through alternative developmental processes, such as regeneration and vegetative reproduction. Regeneration is widespread among animals, and vegetative reproduction (such as budding, fission, and fragmentation) has evolved in about half of the animal phyla. Our main goal is to determine whether embryonic genes, such as homeobox genes, are also involved in these non-embryonic forms of development. We are investigating the expression of homeobox genes during vegetative reproduction and regeneration in annelids, and find preliminary evidence that some developmental pathways are similar between embryogenesis and vegetative reproduction.

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Genetic Recruitment of Body Patterning Genes and Early Life History Diversity of Echinoderms

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Body patterning genes are largely viewed as being developmentally conservative within phyla and even between phyla. However, relatively few comparative studies have been conducted in phyla with complex life histories. We describe the expression of several echinoderm body patterning genes, in a range of species, representing a diversity of larval forms. In echinoderms the developmental roles in some of these genes seems to be quite labile. Differences in the roles of body-patterning genes that are correlated with changes in early life history strategy have also been reported in arthropods. Role recruitment of body-patterning genes is commonly associated with evolutionary changes in morphology, and may be particularly common in phyla with complex life cycles and diversity of life history modes. Generalizations about gene expression and function based on single species, including the highly derived model systems, may not represent typical developmental processes and life histories within their respective phyla.

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NEW ROLES FOR OLD GENES DURING ECHINODERM EVOLUTION

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Echinoderms are among the most distinctive and morphologically diverse of all metazoan phyla. Regulatory genes first characterized in "model" species provide powerful new analytic tools for understanding the developmental bases for the major morphological transformations that occurred during the origin and radiation of echinoderms. We have examined the expression of several transcription factors and signalling molecules in a wide range of echinoderms. Most expression domains in echinoderms occur in structures unique to the phylum; surprisingly few resemble those that are thought to be conserved between arthropods and chordates. Given the phylogenetic proximity of echinoderms to chordates, these results suggest that many regulatory genes have lost conserved developmental roles and gained new roles during echinoderm evolution. Some changes appear to have evolved early in the history of the phylum, and others during the subsequent diversification of larval and adult morphology. These results underscore the evolutionary lability of regulatory genes that are widely viewed as evolutionarily conservative.

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EMBRYOGENESIS WITHOUT EXPRESSION OF THE HOX CLUSTER: TRANSCRIPTS OF MOST HOX GENES APPEAR ONLY IN ADULT BODY PLAN FORMATION IN THE SEA URCHIN LARVA

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Maximally indirect development in sea urchins results in a bilaterally organized, free-living, feeding larva consisting of about 1800 cells. The five-fold radially symmetrical adult body plan is constructed from an imaginal rudiment during the postembryonic or larval phase of existence. The embryonic specification processes rely on short range intercellular signaling and on allocation of invariant lineage elements. It is a prediction of our 1995 evolutionary mechanisms model that the regional specification of morphological components typically mediated by genes of the Hox cluster is not required in this form of embryogenesis. Instead, this mechanism should be required in adult body plan formation. The complete 10-gene Hox cluster of *Strongylocentrotus purpuratus* has now been isolated, mapped and characterized. Here the expression of all but two of these genes is measured quantitatively throughout embryonic and larval development, and in adult tissues. The numbers of molecules of each Hox gene transcript per embryo and per pg of RNA at each stage are calculated from probe excess titrations. Two of these genes (SpHox7 and SpHox10) have previously been shown to be expressed in late embryos; our data confirm this but show, remarkably, that no other Hox gene is expressed significantly through the completion of embryogenesis. Transcripts of these genes are either completely undetectable or are present at insignificant levels of only a small fraction of a molecule per average nucleus. However, once adult body plan formation begins, all of the Hox genes are activated significantly. As an example, SpHox2 was studied by whole mount *in situ* hybridization and is shown here to be expressed, as expected, in a precise five-fold radially symmetric pattern in the imaginal rudiment. All of the Hox genes are also expressed in adult tissues, each in a unique distribution. These observations are consistent with the view that the evolutionary origin of the adult body plans of large metazoans required additional levels of developmental regulatory circuitry, compared to development of small, free-living metazoans of the organizational grade of a modern indirectly developing larva.

1. A NEW OVERVIEW OF SPECIFICATION IN THE EARLY DEVELOPMENT OF THE SEA URCHIN, A TYPICAL TYPE 1 EMBRYO

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Diverse evidence indicates a primordial A/V system built into the egg, which is reflected in differential states of specification during cleavage. This evidence comes from various cis-regulatory analyses and from comparative observations on asteroid echinoderms. The initial conditional specification of endoderm depends both on interblastomers signaling and on the primordial A/V system. Endoderm specification occurs in two steps that may utilize the same genes in different blastomeres. Specification of mesoderm involves activation of genes involving several known transcription factors in the vegetal plate, and also occurs in several stages, and this process is affected by the primordial A/V system as well. A cis-regulatory model postulating parallel cis-regulatory inputs to key endoderm and mesoderm genes has been constructed, and provides an interesting interpretation of LiCl perturbation of endodermal and mesodermal domains.

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An immune system regulatory framework can be imagined, which consists of developmental cis-regulatory addresses that cause activation of genes encoding key transcription factors in immune effector cells, these genes, and the downstream genes are controlled by these factors. Many examples of genes at both levels are known in mammalian immune systems. The rearranging adaptive immunoglobulin and TCR genes are probably recent with respect to deuterostome evolution. New observations on sea urchin immune effector cells show that though these genes are evolutionarily new, appearing only in vertebrate deuterostomes, they have been inserted into an old regulatory framework. Thus some of the transcription factors used to regulate both adaptive and non-adaptive mammalian immune systems, e.g., a specific GATA-2-like factor, a Runt factor, and an inducible NFKB are expressed in sea urchin coelomocytes, and some of the same downstream genes as expressed in lymphoid cells of vertebrates are utilized in coelomocytes, e.g., a C3/4 complement gene, a Factor B (FB) gene, a set of SRCR-like genes, and an HS-1-like gene. The C3/4 and FB genes are linked in the genome, just as in mammals. The homologous feature shared by coelomocytes and mammalian lymphoid cells is essentially the gene regulatory framework.

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EVOLUTION OF ANIMAL BODY PLANS:
A COMPARATIVE GENE EXPRESSION APPROACH TO MESODERMAL PATTERNING IN BASAL DEUTEROSTOMES

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Of considerable importance for understanding the origin and early evolution of bilaterians are the mechanisms underlying the origin and disposition of mesoderm. Two aspects of mesoderm are crucial for an understanding of bilaterian body plans. First, in contrast to the two-dimensional sheets of cells found in "diploblasts", mesoderm allows for the dramatic increase in three-dimensional size found in bilaterians; second mesodermal patterning is an integral component of each phylum's unique body plan. Our primary interest is understanding the early evolutionary history of deuterstomes, the group of bilaterians that includes the chordates, the hemichordates and the echinoderms. Mesodermal components of both chordate (notochord and posterior mesoderm) and echinoderm embryos (secondary mesenchyme) express the transcription factor Brachyury. Comparatively little information is available for the hemichordates, a taxon of key phylogenetic and developmental importance. We examined the expression pattern of Brachyury in conjuction with the formation of embryonic and adult mesoderm in the enteropneust hemichordate *Ptychodera flava*, and compared it with the embryonic and larval expression pattern in the sea urchin *Stronglyocentrotus purpuratus*. The expression pattern in the hemichordate can be divided into three distinct and transient phases:

1) during gastrulation Brachyury is expressed in the future oral and anal regions of the early tornaria larva;

2) after three months larval development, Brachyury is then up-regulated in the gut during the time that mesenchymal cells are ingressing to form the future adult mesocoel and metacoel;

3) during metamorphosis Brachyury is expressed in the mesenchymal cells of the protocoel, mesocoel, and in the very posterior region of the metacoel and gut.

At no time is Brachyury expressed in the stomochord, the putative homologue of the chordate notochord. The sea urchin shows a similar biphasic expression pattern. Initially Brachyury is expressed in the secondary mesenchyme cells of the embryo and thus shows

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little positional similarity with the hemichordate. However, Brachyury is then expressed in the invaginating ectodermal vestibule and in the left and right hydrocoels, the homologoues of the hemichordate left and right mesocoels. Therefore Brachyury is expressed in homologous adult mesodermal components in both echinoderms and hemichordates. Hence, Brachyury is an excellent marker for mesodermal set-aside cells, and an understanding of its cis-regulation and down-stream targets will provide a mechanistic understanding of mesodermal patterning in deuterostomes.

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EVOLUTION OF PATTERN FORMATION IN INSECTS: RADICAL CHANGES IN DEVELOPMENT ARE ASSOCIATED WITH SWITCH IN THE LIFE HISTORY

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An important question in evolutionary developmental biology is how phylogeny and life history interact to affect the course of evolution. Comparative studies indicate that developmental programs are broadly conserved within higher taxa and that fundamental regulatory pathways are often very similar between phyla. Such trends indicate that ancestry obviously plays a significant role in shaping how organisms develop.

On the other hand ecologists and developmental biologists have identified several instances where distinct differences in embryonic development occur between closely related species without any concomitant changes in adult form. If life history plays a role in shaping early development of insects, we would hypothesize that departures from the general insect developmental ground plan would most likely arise in groups whose eggs develop under conditions very different from those experierienced by insects generally. One such group is the parasitic wasps. I will present cellular and molecular evidence that some polyembryonic and monoembryonic wasps violate basic paradigm in insect early development by undergoing complete cleavage of the egg. Characterization of the expression of Drosophila segmentation gene homologs in these wasps revealed drastic changes in the early developmental program relative to Drosophila suggesting that radical changes in development in insects are associated with the switch in life history strategy.

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THE ROLE OF *HOX* GENES IN ESTABLISHING SEGMENT-SPECIFIC TRAITS IN THE LEGS OF *DROSOPHILA*

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In *Drosophila melanogaster Hox* genes control the specific morphology of segments - they break the homonomy of homologous segments to create segments with unique characteristics. For *Hox* genes only rather extreme phenotypes have been identified. Morphological evolution is however believed to occur in a gradual manner. Determining at what levels in the developmental process *Hox* genes are directly controlling segment-specific morphology could reconcile this apparent contradiction.

For the 2nd and 3rd leg of *Drosophila* I want to show how specific bristle patterns are achieved from identical segmental ground plans under the influence of the *Hox* gene *Ubx*. The Ubx expression pattern is dynamic throughout leg development reflecting inputs from the anterior-posterior, proximo-distal and dorso-ventral axes of the leg. In sensory organ precursors the expression of Ubx is often uncoupled from the expression in the surrounding disc epithelium.

Major differences between the bristle pattern of the second and third leg of *Drosophila* are the absence of specific macrochaetes from the third leg: the sternopleural macrochaete, the edge bristle and the apical bristle are missing. Ubx is needed for these differences because in the absence of Ubx function the bristle pattern of the third leg is completely transformed to that of the second leg.

In principle *Hox* genes could interfere with any of the steps leading to bristle development. Different bristles need not be abolished by Ubx in the same way. I found that the apical bristle and the sternopleural macrochaete are affected by Ubx in different ways. The apical bristle precursor appears in all three legs but in the third leg its differentiation is blocked. However, the sternopleural macrochaete precursor is only singled out in the second leg not in the other legs implying that Ubx blocks its development at an earlier stage.

The apical bristle precursor continues to develop after being singled out: it turns on the appropriate gene specifying neuronal type (*cut* which directs an external sensory organ fate) and it undergoes the first round of division . However, shortly before

DEVELOPMENTAL CIS-REGULATORY FUNCTIONS OF OTX AND CONTIGUOUS BINDING SITES IN THE SEA URCHIN EMBRYO

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The orthodenticle-related proteins (Otx) belong to the bicoid class of homeodomain proteins. Previous studies from our lab on the transcriptional regulation of the gut-specific gene Endo16 have revealed that an Otx binding site in module A plays an important role in determining the early vegetal plate and gut expression. William Klein's group has demonstrated that multiple Otx binding sites present in the enhancer region of the aboral ectoderm specific gene Spec2a are both necessary and sufficient for the expression in aboral ectoderm cells but the sequence they used included more than the Otx site. Dominant-negative Otx injected embryos abrogate aboral ectoderm and endoderm formation (Klein's lab preliminary results) suggesting that Otx may play a critical role in both aboral ectoderm and endoderm cell formation. Thus, the paradox is that the same transcription factor is used to specify of two very different cell types. To address the tissue specificity of Otx, we designed oligonucleotide-linked CAT reporter constructs and microinjected them into fertilized eggs. We discovered that a single Otx site either from Endo16 or Spec2a drives CAT expression in endoderm when linked to the basal promoter of Endo16. A different binding site (Y) near the Otx site in the Spec2a enhancer plays an important role in directing expression to the aboral ectoderm when it is associated with an Otx site. These data suggest that interactions among Otx and other transcription factor establish transcriptional selectivity of Otx function, and they address the combinatorial cis-regulatory proprietorial of cell type specificity.

June 10 - 14
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